

SUPPLEMENTARY METHODS

Immunostaining (insulin and glucagon):

Islet cells were fixed with 4% paraformaldehyde in PBS for 15 min and washed once with PBS followed by permeabilization with 0.2% Triton X-100 in PBS at room temperature for 10 min. Cells were then incubated with blocking solution containing 5% bovine serum albumin and 0.1% Triton X-100 in PBS at 37 °C for 30 min or 4°C for overnight. Subsequently, cells were incubated with primary antibodies (1:4000 and 1:200 dilution for mouse anti-glucagon, and rabbit anti-insulin, respectively) for 16 h at 4°C. After washing, cells were stained with the secondary antibodies (AMCA-conjugated donkey anti-mouse IgG or fluorescein-conjugated donkey anti-rabbit IgG). Excitation and emission of AMCA were performed at 345nm and 450nm respectively, while fluorescein was detected using 480nm and 525nm, respectively.

Measurement of ROS by fluorescent probe mitoSOX:

ROS measurements were performed using the mitochondrial superoxide-sensitive dye mitoSOX (Invitrogen). Isolated islets were incubated in 100 μ M STZ for 16 hrs in RPMI-1640 media supplemented with 10% fetal bovine serum and 11mM glucose. Incubations were performed at 37°C and 5% CO₂-95% air. Islets were loaded with 5 μ M mitoSOX for 10 min in KRB buffer solution and washed with fresh buffer afterwards. Fluorescent excitation was achieved at 510nm for 300ms, and emission was detected at 585nm. Experiments were carried out using an Olympus BX51W1 fluorescent microscope fitted with a 20x/0.95 water immersion objective and cooled CCD camera equipped with a magnification changer (U-TVAC, Olympus). A xenon lamp-based DeltaRam high speed monochromator (Photon Technology International) was used for excitation.